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Research Article

Expression of CXCR3 and CXCR4 on Intrahepatic lymphocytes in Normal and Hepatitis C-Infected Liver

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Abstract

Background: During chronic hepatitis C inflammation, several chemokines and chemokine receptors are involved in the accumulation of significant number of lymphocytes in the liver.

Aim: Study the expression patterns of CXCR3 and CXCR4 homing receptors on intrahepatic lymphocyte (IHL) subsets and the frequency of these subsets to understand the mechanism by which these cells migrate to the liver and their roles in the immunopathogenesis of Hepatitis C virus (HCV) infection.

Methods: We investigated the frequencies of $CD3^+$, $CD4^+$, $CD8^+$ and $CD19^+$ lymphocytes, along with chemokine receptors CXCR3 and CXCR4 expression, in paired IHL and peripheral blood (PB)lymphocyte samples of 30 chronic end stage HCV patients and 30 healthy donors by flowcytometry.

Results: Intrahepatic $CD3^+$, $CD8^+$ and $CD8^+$ bright were lower in HCV patients, while $CD8^+$ dim cells were higher. $CD8^+$ dim cells were the only subset to show significantly higher expression of CXCR3 on IHL of chronic HCV patients although all other subsets show insignificant higher expression. On the other hand, CXCR4 expression was significantly lower on most IHL subsets.

Conclusion: The findings of this study provide new information on IHL immunophenotype in chronic end stage HCV infection and suggest that the hepatic trafficking of various IHL subsets may be controlled by different combinations of homing receptors.

Keywords: Chronic HCV, CXCR3, CXCR4, Intrahepatic Lymphocytes

Introduction

Hepatitis C virus is a major cause of chronic liver disease. Chronic infection with HCV can progress to fibrosis, cirrhosis and/or hepatocellular carcinoma [1]. The liver is the primary site of HCV replication and inflammatory response, there by, the study of HCV diseased liver is an important step towards understanding the pathogenesis of HCV. Moreover, a comprehensive characterization of different IHL subsets is crucial for understanding the role of each of these subsets in HCV immunopathogenesis [2].

Immunohistochemical study of liver biopsies has facilitated *in situ* localization of IHL but does not provide details about lymphocyte subtypes or their functional attributes [3]. To fully elucidate the nature of the local immune response in the liver, it is better to study lymphoid cells that are isolated intact and viable [4].

Chemokines and their receptors play an important role in the pathogenesis of HCV infection [5]. Several chemokines and chemokine receptors are considered to be involved in the accumulation of immune cells in the liver. In chronic HCV-infected patients, PB and intrahepatic levels of CXCL9, CXCL10 and CXCL11 chemokines are elevated. Their common receptor, CXCR3, has been detected on most IHL in HCV-infected individuals [6] and its expression tends to increase with the severity of liver inflammation [2]. CXCR3 induces lymphocyte recruitment from hepatic sinusoids to liver parenchyma [7]. It is highly expressed in activated T lymphocytes. CXCR3 belongs to Th1 cell surface chemokine receptors, which is abundantly distributed in HCV-infected liver lymphocytes [8] and causes Th1 initiated tissue damage. Furthermore, it was found that gene polymorphism at CXCR3 gene locus is one of factors contributing to chronicity in HCV patients [9].

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CXCL12, also known as stromal-derived factor 1 alpha, is the specific ligand for CXCR4 [10]. Hepatic expression of CXCL12 is induced in response to proinflammatory stimuli [11]. The role of CXCL12 and its receptor CXCR4 in the recruitment and retention of immune cells in the liver during chronic HCV has been studied by Wald et al. who proposed that over 50% of liver infiltrating lymphocytes express CXCR4 in response to CXCL12 [12].

The study of factors related with clinical prognosis after HCV infection is of critical importance for predicting disease progression of HCV infection and for providing new treatment strategy. Therefore, in this study, we investigated the frequencies of major lymphocyte subsets $CD3^+$, $CD4^+$, $CD8^+$ and $CD19^+$ cells in IHL and PB lymphocytes of HCV infected patients compared to healthy donors. We also provided a comparative analysis of chemokine receptor CXCR3 and CXCR4 expression on different lymphocyte subsets to examine the potential role of these chemokines in recruiting immune cells to the liver during chronic HCV infection.

Materials and Methods

Patients and specimens

Liver tissue samples were obtained within one hour of explantation from 30 patients with end stage chronic HCV infection undergoing liver transplantation in Ain Shams University specialized hospital. All patients were anti-HCV antibody positive (Architect) and HCV-RNA positive by polymerase chain reaction (Amplicor HCV, Roche Diagnostic Systems). All patients were negative for hepatitis B surface antigen and anti-HIV antibody and markers of autoimmune hepatitis (autoantibodies titre <1/40). Liver tissue was obtained from organs removed from recipients at the time of liver transplantation. Liver tissue was collected in RPMI 1640 (Lonza). Perfusate of donated liver was obtained at the time of transplantation and has been used as source of IHL for thirty healthy donors [13]. Matched PB samples (6 ml) were obtained at the time of transplantation from both recipient and donor. Written informed consent was obtained from each patient included in the study and the study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected by the approval of the ethical committee of Faculty of Medicine, Ain Shams University.

Isolation of IHL from explanted liver by mechanical and enzymatic method

A variety of methods have been attempted for IHL isolation and purification, of which we used the modified enzymatic dispersal protocol. Liver tissue specimens were gently teased apart into $1mm^3$ small pieces. The small liver tissue fragments were injected with 40 ml warm HBSS (37ºC) (Gibco, Life Technologies) containing collagenase IV (0.5 mg/ml (Invitrogen, Life Technologies), DNase I (50 ng/ml) (Invitrogen, Life Technologies), FCS (2%) (Lonza, BioWhittaker) and BSA (Sigma -Aldrich) (0.6%) directly into the tissue aiming to loosen it. Then, the tissue was incubated at 37ºC for 15 min with frequent shaking. The tissue suspension was filtered through a 100 µm cell strainer (BD Bioscience) then washed and finally IHL were isolated using Ficoll-Hipaque (Lonza, BioWhittaker) density gradient centrifugation followed by washing twice with RPMI 1640 supplemented with 2mM l-glutamine, 25mM HEPES, 100 U/mL benzylpenicillin, 0.1mg/mL streptomycin, and 10% AB serum (complete medium) (Lonza, BioWhittaker) before staining by monoclonal antibodies. Cell viability was assessed utilizing Guava ViaCount FlexReagent for Flow Cytometry (Merck Millipore, France) to ensure >95% viability.

Isolation of IHL from perfusate of donated liver

Briely, a 50 ml aliquot of donated liver perfusate was taken at the time of transplantation. Liver perfusate was layered over Ficoll-Hipaque to isolate lymphocytes which were then washed twice in complete medium before staining by monoclonal antibodies.

Isolation of peripheral blood mononuclear cells (PBMCs)

PBMCs were isolated from 6 ml Heparin-anticoagulated blood samples using Ficoll-Hipaque density gradient centrifugation. After isolation, cells are washed twice in complete medium before staining by monoclonal antibodies.

Cell staining and flow-cytometric analysis

Freshly isolated IHL or PBMCs were resuspended (10^6 cells per ml) in FACS buffer and stained. All antibodies were purchased from BD Pharmingen except CD3 (eBioscience, San Diego, CA, USA). Antibodies used included CD3-FITC, CD8-PECy7, CD4-PECy5, CD19-PECy5, CXCR3-PE and CXCR4-PE. Appropriate isotype-matched monoclonal antibodies were used to establish gating parameters (Figure 1). Data was collected using a four-colour Guava cytometer (Merck Millipore, France) and analysis was performed using FlowJo software (TreeStar, La Jolla, USA).

Statistical analyses

Data was analyzed using Prism 5 software (GraphPad, La Jolla, CA). Patient and control groups were compared using a two-tailed Mann–Whitney test. Correlations between parameters were determined using Spearman's correlation coefficient.

Results

Using flowcytometry, we investigated the frequency of different lymphocyte subsets as well as CXCR3 and CXCR4 expression in liver and blood samples from patients with chronic HCV infection and healthy subjects.

Baseline Characteristics of Chronic end stage HCV Patients

Thirty chronic end stage HCV patients were enrolled. Demographical and clinical characteristics of patients are reported in Table 1.

Lymphocyte subsets in the peripheral blood and the liver of chronic HCV patients and healthy donor

The frequencies of $CD19^+$, $CD3^+$, $CD4^+$, $CD8^+$, $CD8^+$ bright, $CD8^+$ dim, $CD4^+CD8^+$ double positive T cells (DPT) and $CD4^-CD8^-$ double negative T cells (DNT) cells in the liver and blood of patients with chronic HCV infection and healthy donors were analyzed. Analysis showed a statistically significant difference between chronic HCV IHL and healthy IHL regarding $CD3^+$ and $CD8^+$ cells which were lower in chronic HCV patients than in the healthy subjects with P value < 0.001. Meanwhile no significant difference was observed in $CD3^+$ and $CD8^+$ between IHL and PB in chronic HCV

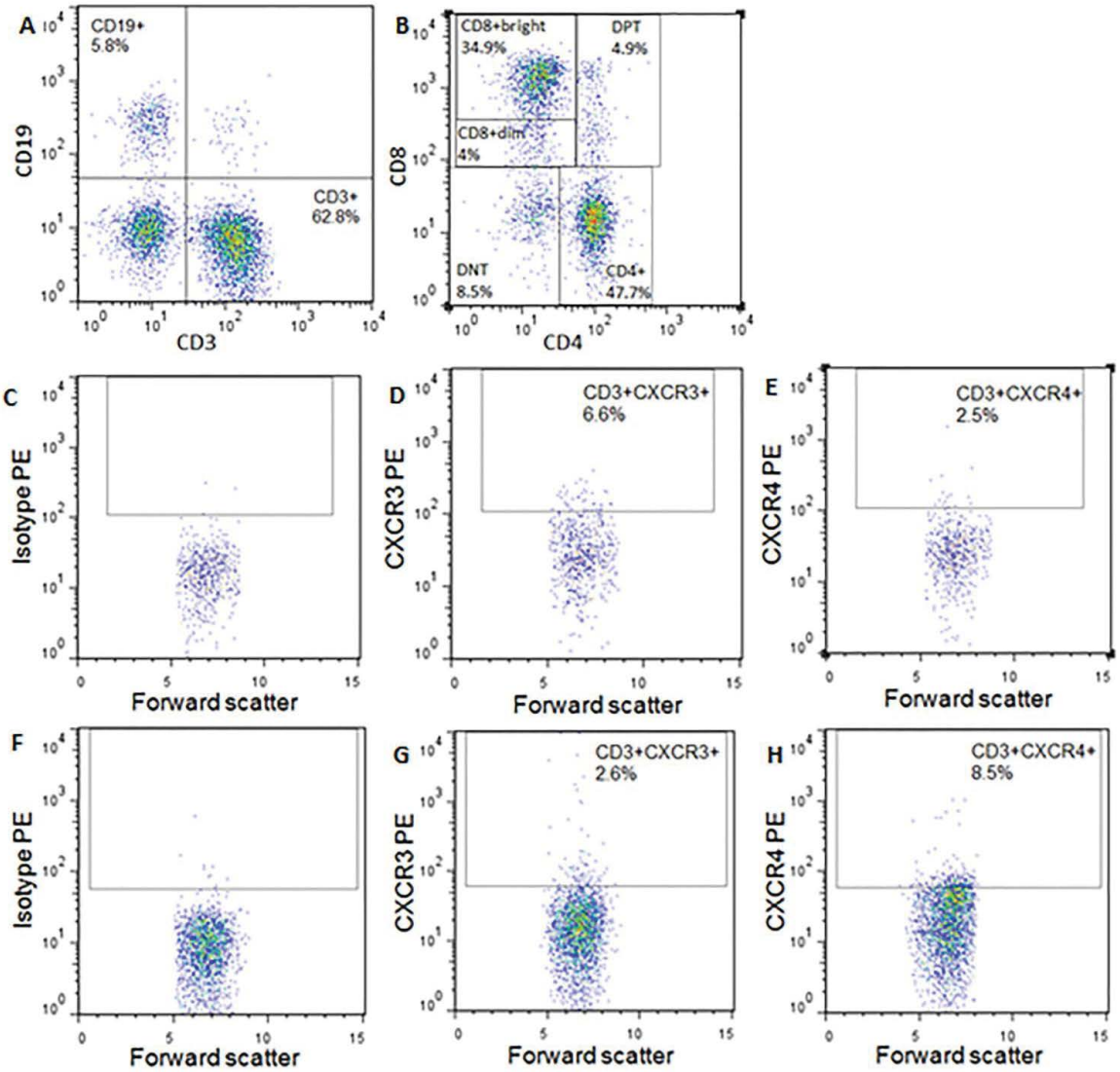

Figure 1: Representative flowcytometry plots and gating of intrahepatic lymphocytes.
(A) shows gating strategy for the percentage of $CD3^+$ and $CD19^+$ cells in chronic HCV patient.
(B) shows gating strategy for the Percentage of $CD4^+$, $CD8^+$ bright, $CD8^+$ dim, DPT and DNT cells in chronic HCV patient.
(C) Representative Chronic HCV patient results for $CD3^+$ IsoPE
(D) Representative Chronic HCV patient results for $CD3^+$ $CXCR3^+$
(E) Representative Chronic HCV patient results for $CD3^+$ $CXCR4^+$
(F) Representative Healthy donor $CD3^+$ IsoPE
(G) Representative Healthy donor $CD3^+$ $CXCR3^+$
(H) Representative Healthy donor $CD3^+$ $CXCR4^+$

Table 1: Demographical and clinical characteristic of chronic HCV patients Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; IQR, the interquartile range; HCV, hepatitis C virus.

	Chronic HCV patients
Age, median (range)	52(43-68)
Gender	
Male, *n*	27
Female, *n*	3
ALT (IU/ml), median (IQR)	50 (32-55)
AST (IU/ml), median (IQR) Total bilirubin (mg/dL), median (IQR)	54 (30-70) 2.2 (1.3-4.5)
HCV RNA(IU/ml), median (IQR)	205000 (65000-1890000)
Total leukocyte count (10^9/L), median (IQR)	4.1 (1.5-9.0)

patients. Also, no significant difference was observed between PB of patients and controls concerning $CD3^+$ and $CD8^+$ cells (Table 2 and Figure 1).

The frequency of IHL $CD8^+$ dim subset of chronic HCV patients was higher than those of healthy subjects and was also higher than their PB. Whilst intrahepatic $CD8^+$ bright cells were lower in HCV patients than healthy subjects (P value < 0.001).

IHL showed skewing of the $CD4^+/CD8^+$ ratio towards $CD8^+$ lymphocytes with a ratio of 1:1.28 in chronic HCV patients and a ratio of 1:2 in healthy donors. In chronic HCV patients, $CD4^+$ cells were significantly higher in the PB than in IHL (P value < 0.001). Meanwhile, chronic HCV IHL $CD4^+$ cells were higher than

Table 2: Median percentage of lymphocyte subsets in the peripheral blood and the liver of chronic HCV patients and healthy donors
Abbreviations: IHL, intrahepatic lymphocyte; HD, healthy donors; PBMCs, peripheral blood mononuclear cells.

	cHCV IHL	HD IHL	*p*	cHCV IHL	cHCV PBMCs	*p*	cHCV PBMCs	HD PBMCs	*p*
$CD19^+$	3.1	5.7	0.7405	3.1	6.3	0.8315	6.3	7.9	0.4375
$CD3^+$	30	53	0.0001	30	20	0.0757	20	26	0.1380
$CD4^+$	32	25	0.171	32	49	< 0.0001	49	49	0.6421
$CD8^+$	41	51	0.001	41	38	0.2608	38	34	0.8703
$CD8^+$bright	51	76	0.0001	51	66	0.0518	66	83	0.05
$CD8^+$dim	49	24	0.0001	49	30	0.0127	30	16	0.0959
$CD4^+CD8^+$	4.0	2.4	0.0347	4.0	1.9	0.0001	1.9	2.8	0.1278
$CD4^-CD8^-$	16	15	0.4880	16	9.4	0.001	9.4	12	0.5687

Table 3: Median percentage of intrahepatic and peripheral lymphocyte subsets expressing CXCR3
Abbreviations: IHL, intrahepatic lymphocyte; HD, healthy donors; PBMCs, peripheral blood mononuclear cells.

	cHCV IHL	HD IHL	*p*	cHCV IHL	cHCV PBMCs	*p*	cHCV PBMCs	HD PBMCs	*p*
$CD19^+$	2.9	3.5	0.5845	2.9	1.9	0.4644	1.9	2.1	0.6184
$CD3^+$	4.1	2.3	0.2182	4.1	7.3	0.5949	7.3	6.2	0.5806
$CD4^+$	6.8	5.0	0.9175	6.8	7.8	0.9758	7.8	5.3	0.9470
$CD8^+$	2.6	1.6	0.2804	2.6	6.7	0.1674	6.7	4.5	0.1406
$CD8^+$bright	5.1	2.0	0.1808	5.1	10	0.5231	10	7.4	0.5318
$CD8^+$dim	6.4	2.3	0.0063	6.4	6.5	0.7496	6.5	8.0	0.4551
$CD4^+CD8^+$	6.5	2.2	0.2300	6.5	6.7	1.0000	6.7	8.0	0.6999
$CD4^-CD8^-$	3.8	3.3	0.1813	3.8	4.7	0.7266	4.7	3.4	0.7033

IHL $CD4^+$ in healthy donors, however the difference was not statistically significant.

The patterns of DPT cells in our study showed significant elevation in chronic HCV IHL compared to their PB counterparts and to healthy donor IHL ($p < 0.0001$ & < 0.05 respectively).

As for $CD4^-CD8^-$ DNT-cells, they were significantly higher in chronic HCV IHL compared to their PB (*P* value < 0.001). We also noticed that these IHL cells were higher in patients than healthy donor however the frequency didn't significantly differ.

Expression of CXCR3 and CXCR4 on lymphocyte subsets

The expression of CXCR3 was higher on all HCV IHL subsets (except for $CD\ 19^+$Cells) compared to healthy IHL, however the difference didn't reach significance except for CD8 dim cells ($p < 0.01$). Moreover, no significant difference was observed upon comparing IHL with the PB subsets (Table 3, Figure 2).

To examine the potential of the CXCR4/CXCL12 pathway to mediate extravasation of cells from the circulation into inflamed tissue, we assessed the expression of CXCR4, which was significantly lower on all IHL subsets of chronic HCV patients compared to healthy donors (except for $CD8^+$dim and $CD8^+$ bright subsets the difference didn't reach significance). CXCR4 expression was also lower in chronic HCV IHL compared to PB counterparts except for $CD4^+$ which was nearly significant (P = 0.055) (Table 4, Figure 3).

Correlation between different intrahepatic lymphocyte subsets, ALT, viral load and serum bilirubin

We did not find any statistically significant correlation between any IHL subset and the HCV RNA level. A significant negative correlation was observed between DNT and both serum ALT and bilirubin levels. On the other hand, $CD8^+$ cells showed a significant positive correlation with both of ALT and bilirubin

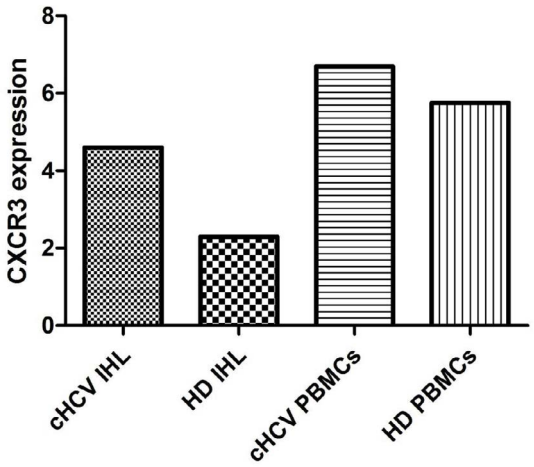

Figure 2: Percentage of intrahepatic and peripheral blood lymphocytes expressing CXCR3.

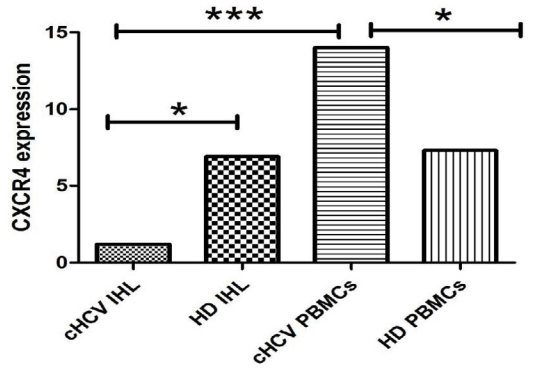

Figure 3: Percentage of intrahepatic and peripheral blood lymphocytes expressing CXCR4.
∗ indicates $P < 0.05$; and ∗∗∗ indicates $P < 0.001$.

Table 4: Median percentage of intrahepatic and peripheral lymphocyte subsets expressing CXCR4
Abbreviations: IHL, intrahepatic lymphocyte; HD, healthy donors; PBMCs, peripheral blood mononuclear cells.

	cHCV IHL	HD IHL	*p*	cHCV IHL	cHCV PBMCs	p	cHCV PBMCs	HD PBMCs	*p*
$CD3^+$	1.2	4.4	0.0297	1.2	13	0.0016	13	5.4	0.1694
$CD4^+$	1.6	14	0.0161	1.6	14	0.0557	14	6.0	0.4625
$CD8^+$	2.5	7.7	0.0200	2.5	16	0.0002	16	7.3	0.1816
$CD8^+$bright	1.0	10	0.0711	1.0	22	0.0005	22	6.6	0.0396
$CD8^+$dim	2.6	4.9	0.1817	2.6	8.6	0.0106	8.6	8.8	0.8841
$CD4^+CD^8$+	0.65	6.9	0.0187	0.65	18	0.0051	18	12	0.5433
$CD4^-CD8^-$	0.90	5.0	0.0018	0.90	9.7	0.0004	9.7	10	0.6694

Table 5 Correlations between different lymphocyte subsets, ALT, viral load and total bilirubin.
Abbreviations: ALT, alanine aminotransferase.

	ALT		Viral Load		Total bilirubin	
	Rs	*p*	Rs	*p*	Rs	p
$CD19^+$	0.61	0.045	-0.46	0.1501	0.20	0.557
$CD3^+$	0.46	0.071	-0.30	0.258	0.34	0.196
$CD4^+$	0.12	0.674	0.17	0.552	-0.29	0.316
$CD8^+$	0.53	0.040	0.02	0.929	0.61	0.016
$CD8^+$bright	-0.006	0.986	-0.16	0.662	0.25	0.491
$CD8^+$dim	0.006	0.986	0.16	0.662	-0.25	0.491
$CD4^+CD8^+$	0.12	0.698	0.36	0.222	0.67	0.012
$CD4^-CD8^-$	-0.63	0.012	0.02	0.939	-0.56	0.031
CXCR3						
$CD19^+$	0.32	0.330	-0.24	0.484	0.67	0.030
$CD3^+$	0.27	0.414	0.12	0.729	0.67	0.027
$CD4^+$	0.19	0.575	0.24	0.474	0.73	0.012
$CD8^+$	0.21	0.454	0.13	0.647	0.64	0.009
$CD8^+$bright	0.17	0.638	-0.15	0.676	0.75	0.017
$CD8^+$dim	0.00	1.00	-0.15	0.672	0.68	0.034
$CD4^+CD8^+$	0.30	0.400	0.02	0.946	0.61	0.066
$CD4^-CD8^-$	0.10	0.768	-0.11	0.749	0.54	0.087
CXCR4						
$CD3^+$	-0.05	0.877	-0.11	0.738	0.03	0.924
$CD4^+$	-0.03	0.899	-0.28	0.351	0.34	0.250
$CD8^+$	-0.21	0.497	-0.07	0.816	0.17	0.588
$CD8^+$bright	-0.06	0.853	0.04	0.893	0.49	0.154
$CD8^+$dim	-0.46	0.186	0.18	0.622	0.18	0.632
$CD4^+CD8^+$	-0.42	0.225	-0.01	0.959	-0.54	0.113
$CD4^-CD8^-$	0.01	0.971	-0.12	0.705	0.75	0.003

levels and DPT showed a significant positive correlation with bilirubin levels. Most of the $CXCR3^+$ IHL, with the exception of DPT and DNT cells, showed a significant positive correlation with bilirubin levels (Table 5).

Discussion

HCV has an extraordinary capacity to subvert the immune response enabling it to establish chronic infections. Several chemokines and chemokine receptors are thought to be involved in the recruitment and activation of immune cells in HCV-inflamed liver [14]. HCV is known to modulate chemokine expression and may therefore enable its survival by subverting the immune response through altered leukocyte chemotaxis resulting in impaired viral clearance and establishment of chronic low-grade inflammation [11].

In the current study, IHL $CD8^+$ and $CD8^+$ bright cells were found to be lower in chronic HCV patients than in the healthy controls. This relative scarcity of circulating $CD8^+$ T effector cells has been documented [15] and may contribute to HCV persistence in the liver Moreover, it was found that chronic HCV infections are associated with transient delayed responses that are weak and target a narrow range of MHC class I and II restricted epitopes [16], Such impairment might give rise to insufficient T-cell priming and expansion of HCV-specific clones [17] which may give a plausible explanation for the apparent deficiency of patients' $CD8^+$ and $CD8^+$ bright cells in the current study.

In comparison to PB, the most noteworthy difference is the skewing of the IHL $CD4^+/CD8^+$ ratio towards $CD8^+$ lymphocytes and the elevated PB $CD4^+$ cells compared to IHL in both donors and HCV patients. Immune cells have been reported to be susceptible for HCV replication, the expression of HCV core protein in CD4 T cells increases the proliferative potential of CD4 cells [18]. Meanwhile, we observed that HCV IHL $CD4^+$ cells were higher than healthy IHL $CD4^+$ (although the difference wasn't significant). Deignan et al reported similar results; they found significant $CD4^+$ T cells expansion in livers with chronic HCV [19].

For DPT, the presence in the liver of cells expressing both CD4 and CD8 co-receptors could result from the accelerated recruitment of precursors from the bone marrow followed by their rapid development towards a T cell phenotype in the thymus [20]. It could be a compensatory mechanism for the loss of terminally differentiated T lymphocytes resulting from the chronic infection or, more directly, viral factors [21]. Our results regarding DPT IHL are in accordance with these publications.

The present study also provides insight into DNT cells. We explained the increased proportion of DNT IHL cells in HCV patients on the basis that $CD4^-$ $CD8^-$ DNT cells have been involved in the mechanisms of hepatitis viral persistence, it has been postulated that the DNT cells are able to kill virus-specific $CD8^+$ T cells via the Fas/FasL dependent pathway [22]. This in turn explains the significant negative correlation between DNT cells and both serum ALT and bilirubin meanwhile $CD8^+$ cells are positively correlated with both of them.

One feature of our data was the study of differential expression of chemokine receptors CXCR3 and CXCR4 on the major lymphocyte subsets. CXCR3, is known to be predominantly expressed on activated Th-1 cells and plays an important role in recruiting both activated Th1 and CD8 T cells to sites of infection [18]. Up regulated surface expression of CXCR3 on liver infiltrating CD4 effector lymphocytes and intrahepatic memory T cells has been reported in the livers of HCV-infected individuals [23]. Confirming prior observations, we detected higher expression of CXCR3 on all HCV IHL T cell subsets ($CD3^+$, $CD8^+$, $CD4^+$, DPT

and DNT) compared to donor IHL T cell subsets. Moreover, most of $CXCR3^+$ IHL with the exception of DPT and DNT cells showed positive correlation with serum bilirubin. It has been reported that during chronic hepatitis C, there is an increased intra-hepatic production of chemokines that bind to the CXCR3 receptor including CXCL9, IP-10 or CXCL10 and CXCL11 [24]. This in turn increase the migration of CXCR3 IHL towards CXCL9 and CXCL10 released by sinusoidal ECs and hepatocytes [25].

Our results demonstrate that the frequency of chronic HCV IHL $CXCR3^+$ T cells is lower than the circulating counterparts, (again the difference wasn't significant). It is possible that due to decreased proportions of $CXCR3^+$ T cells, CXCL10 is upregulated in an attempt to recruit more $CXCR3^+$ T cells to the liver. The elevated CXCL10 concentration could disrupt the chemokine sensing gradient, and as a result $CXCR3^+$T cells migrate away from the site of infection "chemorepulsion" [26,27] resulting in downregulation of CXCR3 expression in response to overstimulation from a high ligand concentration [5].

According to their anatomical distribution, CXCL12 and its receptor CXCR4 may be involved in directing immune cells from the circulation to the liver, and in promoting their retention in the fibrotic liver. CXCL12 is thus important in the organization of tissues following damage [12]. By investigating the trafficking patterns of CXCR4 expressing cells, we detected significant lower CXCR4 expression on HCV IHL T cells compared to both donor IHL T cells and PB T lymphocytes. This could be attributed to CXCR4 down-regulation on naive T cells following T cell priming and differentiation to memory/effector T cell or could be due to chronic elevated level of inflammatory cytokines [28]. Previous reports have indicated down-regulation of CXCR4 upon activation of T, NKT and NK cells with IL-2 or IL-15, and up-regulating the cell surface expression of CXCR3. The decreased expression of CXCR4 may promote mobilization of the cells from hematopoietic organs to the PB [29,30]. Contradictory results have demonstrated high expression levels of CXCR4 on IHL in chronically HCV inflammed liver. They attributed the induction of CXCR4 to TGF-β which is involved in the retention of only recently activated lymphocytes in the liver [12].

The current study revealed higher frequency of intrahepatic $CD19^+$ $CXCR3^+$ cells in comparison to PB $CD19^+$ $CXCR3^+$ cells in chronic HCV patients (although the difference wasn't significant). Many reports indicate that IgG plasma-cell precursors formed in a secondary immune response against systemic antigen migrate toward ligands for CXCR3, likely allowing them to migrate to inflamed tissue [31].

To conclude, our results indicate that the degree of inflammation and the type of immune cells infiltrating the liver during chronic end stage HCV infection are affected by the differential and coordinated expression of several chemokines. Some chemokines, mainly the Th1 chemokine CXCL10 and its receptor CXCR3 are up-regulated during HCV infection whilst CXCR4 are downregulated during the course of disease progression. Such manipulation of chemokine expression and receptor–ligand interactions by HCV is likely to favor subversion of antiviral immunity and chronicity resulting in persistent inflammation and hepatic tissue damage. Finally, it may be worthwhile exploring new therapeutic interventions based on the role of these homing receptors in chronic HCV infection.

Abbreviations

Alanine aminotransferase (ALT), aspartate aminotransferase (AST),Double negative T cells (DNT),Double positive T cells (DPT) ,Hepatitis C virus (HCV) ,dipeptidylpeptidase (DPP) .

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Conflict of Interest

All authors declare that they have no financial or commercial conflicts of interest.

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